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Research Article

Simultaneous Determination of Four Ingredients in *Plantago Depressa* by Single Marker

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Objective. To establish a quantitative analysis of multicomponents by single marker (QAMS) method for the simultaneous determination of 4 active components such as protocatechuic acid, catechin, quercetin, and luteolin in *Plantago depressa*. *Method*. 4 active components in *Plantago depressa* were studied. Quercetin was used as an internal reference to establish the relative correction factors among protocatechuic acid, catechin, and luteolin and calculate the contents of each component; the results were compared with those measured by the external standard method. *Results*. 4 components showed a good linear relationship in their respective concentration ranges ($r > 0.9995$). The relative correction factors (fs/k) of protocatechuic acid, catechin, and luteolin were 1.1992, 0.8613, and 1.6069, respectively. The method had good durability. The contents of protocatechuic acid, catechin, and luteolin calculated by QAMS were not significantly different from those measured by the external standard method. *Conclusion*. QAMS can be used to determine the content of 4 components in *Plantago depressa* at the same time, and the method is simple, accurate, and can be used for quality control.

1. Introduction

Plantago depressa is recorded in the Chinese Pharmacopoeia (2015 edition) [1], which is the dried whole plant of *Plantago asiatica* L. or *Plantago depressa* Wild., with the effects of clearing heat, promoting diuresis, relieving stranguria, eliminating phlegm, cooling blood, and detoxifying. It can be used for treating heat stranguria, difficulty and pain, edema, oliguria, summer humidity, alvine flux, phlegm, heat cough, carbuncles, etc. *Plantago depressa* has a long history, and it is widely used in China. Whether it is a single medicinal material or a compound preparation [2], it has a certain clinical application market. The main chemical components of *Plantago depressa* include flavonoids [3–7], phenylethanoid glycosides [6,7], iridoid terpenoids, triterpenoids, and sterols [4–7]. According to the literature on the quality standard of *Plantago depressa*, the single component is mainly used as the evaluation index of the quality of *Plantago depressa*, which is contradictory to the characteristics of the multicomponent synergistic effect of traditional Chinese medicine. In the previous studies of this subject, it was found that protocatechuic acid, catechin, quercetin, and luteolin contained in *Plantago depressa* had not been reported for content evaluation, and these four components had remarkable effects in antibacterial activity [8–13], detumescence, anti-inflammatory [10–13], eliminating phlegm, antivirus [11], and protecting the liver and gallbladder [14–16], which indicates that it is of great significance to detect the contents of protocatechuic acid, catechin, quercetin, and luteolin in *Plantago depressa*.

Quantitative analysis of multicomponents by single marker (QAMS) refers to a method that utilizes the relationship between internal functions and proportions of effective components of traditional Chinese medicine and realizes the synchronous determination of multicomponents (reference substances are difficult to obtain or supply) by measuring one component (reference substances are cheap or easy to obtain) [17], and it is a multi-index

quality evaluation model suitable for the characteristics of traditional Chinese medicine [18, 19]. QAMS method is an effective method for multi-index quality control. [20, 21]. The HPLC method has high sensitivity, fast analysis speed, and high separation efficiency [22, 23]. In the Pharmacopoeia (2010 edition), the QAMS method was adopted for the first time to establish the medicinal standard of *Coptis chinensis*, and the development direction of "single index-multi-index, index component -pharmacological component control" was established, which was widely recognized in the industry [24, 25]. In this paper, the QAMS method for the simultaneous determination of protocatechuic acid, catechin, and luteolin (Figure 1) in *Plantago depressa* was established by using quercetin, a cheap and readily available substance, as the internal standard, which laid the foundation for the establishment of its quality standard.

2. Instruments and Materials

Chromatographic analysis was performed on Agilent 1260 high-performance liquid chromatography system (including a quaternary low pressure mixing pump, autosampler, column oven, 1100 diode array detector, and ChemStation workstation); ALC-110.4 millionth electronic balance was purchased from Beijing Sardolis Instrument System Co., Ltd.; KQ-250 ultrasonic cleaner was obtained from Kunshan Ultrasonic Instrument Co., Ltd.; and DFY-200 (swing type) multifunction high-speed traditional Chinese medicine grinder was purchased from Shanghai Zule Instrument Co., Ltd..

The protocatechuic acid (batch number: 110809–201205, purity of 99.9%), catechin (batch number: 110877–201604, purity of 99.2%), quercetin (batch number: 100081–201610, purity of 99.1%), and luteolin (batch number: 111520–201605, purity of 99.6%) were purchased from the China Pharmaceutical Biological Products Verification Institute (Beijing, China). Methanol (Fisher, America) was of chromatographic grade; ultrapure water was acquired from Hangzhou Wahaha Co., Ltd.; and other reagents were all of analytical grade.

The source information of 10 batches of *Plantago depressa* is shown in Table 1. It was identified as the dried whole plant of *Plantago depressa* L. or *Plantago depressa* Wild. by associate professor Xiao Jinglei from the Department of Traditional Chinese Medicine Identification of Changchun University of Chinese Medicine.

3. Experimental Methods

3.1. Chromatographic Condition. Phenomenex C_{18} column (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of methyl alcohol(A) and 0.1% phosphoric acid solution (B), gradient elution (0 ~ 7 min, 5%A; 7 ~ 10 min, 5%⟶15%A; 10 ~ 20 min, 15%A; 20 ~ 25 min, 15%⟶31%A; 25 ~ 32 min, 31%A; 32 ~ 40 min, 31%⟶47%A; and 40 ~ 58 min, 47%⟶56%A). The flow rate was 1.0 mL·min^{-1}; the detective wavelength was monitored at 225 nm; the column was maintained at a temperature of 30°C; and the injection volume was 10 μL.

3.2. Preparation of Solution

3.2.1. Reference Solution. The proper amounts of protocatechuic acid and catechin were taken and weighed precisely, and methanol was added to prepare a solution containing 0.74 mg/mL protocatechuic acid and 0.678 mg/mL catechin, respectively; the proper amounts of quercetin and luteolin were taken, weighed precisely, and placed in a 25 mL flask. 6.25 mL of the above two solutions were added, and methanol was added to prepare a mixed reference substance mother solution containing 0.185 mg/mL protocatechuic acid, 0.1695 mg/mL catechin, 0.8232 mg/mL quercetin, and 0.442 mg/mL luteolin. 1 mL mixed reference substance mother solution was precisely sucked and put into a 50 mL flask, and methanol was added to prepare a mixed reference substance solution containing 3.70 μg/mL protocatechuic acid, 3.39 μg/mL catechin, 16.46 μg/mL quercetin, and 8.84 μg/mL luteolin.

3.2.2. Solutions for Testing Products. The proper amount of dried *Plantago depressa* herb was taken, pulverized, weighed (about 5 g) precisely, and put into a conical flask with a cover. After 8 times of methanol was added, ultrasound (250 W, 40 kHz) was used to treat it for 30 min, cooling it, filtering it, repeating treatment twice, combining the filtrates, recycling the solvent rotationally, and transferring it to a 5 mL flask, adding methanol to fix the volume to the scale. Finally, the product can be obtained.

3.3. Methodological Investigation

3.3.1. Investigation of Linear Relationship. 0.02, 0.04, 0.1, 0.2, 0.04, and 1.0 mL of mixed reference substance mother solution were accurately sucked under the item "2.2.1," and they were placed in 5 mL measuring flasks, respectively. Methanol was added to dilute to the scale, shaking well. A mixed reference substance solution with different mass concentrations was prepared. 10 μL of each of the above 6 solutions were sucked, respectively, and injected into high-performance liquid chromatography (HPLC). Taking the concentration as an abscissa (X) and the peak area integral as an ordinate (Y), the standard curve was drawn, and the regression formula was calculated.

3.3.2. Precision Test. 10 μL of the same mixed reference substance solution was precisely sucked and injected into high-performance liquid chromatography, and the chromatographic peak area was recorded 6 consecutive times.

3.3.3. Repeatability Test. 6 *Plantago depressa* (No. ZJ-1) from the same batch were taken, and 6 solutions for testing the product in parallel were prepared according to the method under item "2.2.2." 10 μL of each solution was precisely sucked and injected into high-performance liquid chromatography, and the chromatographic peak area was recorded.

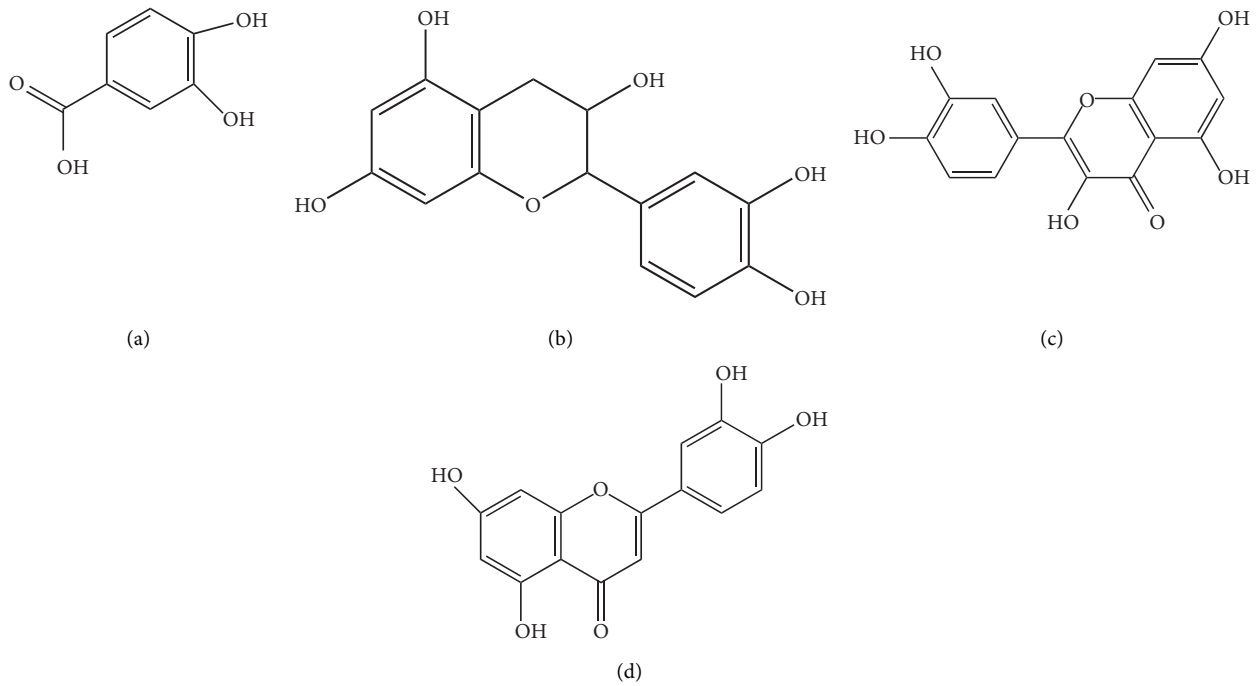


Figure 1: (a) Protocatechuic acid, (b) catechin, (c) quercetin, and (d) luteolin.

3.3.4. Stability Test. Precision absorption of the same sample solution (No. ZJ-1) 10 μL of each was injected into a high-performance liquid chromatography at 0, 2, 4, 6, 8, and 12 h after preparation, and the chromatographic peak area was recorded.

3.3.5. Sample Addition Recovery Test. 6 *Plantago depressa* sample powders with known content were taken, each copy was about 2.5 g, weighing precisely, adding 20 μL of 0.502 mg/mL protocatechuic acid solution, 20 μL of 0.455 mg/mL catechin solution, 100 μL of 0.412 mg/mL quercetin solution, and 50 μL of 0.502 mg/mL luteolin solution, respectively. Methanol was added to 20 mL, and then the solution for testing the product was prepared according to the method under item "2.2.2" for sample analysis.

3.4. Calculation of Relative Correction Factor f. The mixed reference substance mother solution prepared was taken under item "2.2.1," diluting it by the corresponding multiple, and injecting 10 μL, respectively. Using quercetin as an internal reference, the relative correction factors of quercetin to protocatechuic acid, catechin, and luteolin were calculated according to formula (1), respectively.

$$fsi = \frac{fs}{fi} = \frac{(As/Cs)}{(Ai/Ci)}. \quad (1)$$

As is the peak area of the reference substance, *Cs* is the concentration of the reference substance, *Ai* is the peak area of other components, and $\underline{Ci}$ is the concentration of other components.

3.5. Investigation of Durability

3.5.1. Influence of Different Instruments and Chromatographic Columns on f. The effects of different chromatographic columns and chromatographic systems on the relative correction factors were investigated, and the RSD of the relative correction factors of different chromatographic columns and chromatographic systems was calculated.

3.5.2. Influence of Different Volume Flowrates on f. The effects of different volume flow rates (0.8, 0.9, 1.0, 1.1, and 1.2 mL/min) on the relative correction factors were investigated, and the RSD of the relative correction factor under different flow rates was calculated.

3.5.3. Influence of Different Column Temperatures on f. The influence of different column temperatures (20, 25, 30, 35, and 40°C) on the relative correction factor was investigated, and the RSD of the relative correction factor under different column temperatures was calculated.

3.6. Location of Chromatographic Peaks of Components to be Detected. Using quercetin as an internal reference, the relative retention method was used to locate protocatechuic acid, catechin, and luteolin, respectively. The effects of the Agilent 1260 and Shimadzu LC-2030 high-performance liquid chromatograph and the Agilent ZORBAX SB-C_{18}, Phenomenex C_{18}, and Agilent TC-C_{18} columns on the relative retention value were also investigated.

$$Rt_{si} = \frac{t_{Ri}}{t_{Rs}}. \quad (2)$$

Table 1: Comparison of 4 components in *Plantago depressa* from different sources (μg/g).

Number	Sources	Quercetin	Protocatechuic acid			Catechin			Luteolin		
			ESM	QAMS	RSD/%	ESM	QAMS	RSD/%	ESM	QAMS	RSD/%
ZJ-1	Jinhua, Zhejiang	16.755	3.753	3.791	0.71	3.387	3.426	0.81	8.749	8.635	0.93
ZJ-2	Jinhua, Zhejiang	13.432	2.117	2.175	1.91	2.365	2.387	0.65	10.853	10.741	0.73
ZJ-3	Jinhua, Zhejiang	15.491	4.142	4.241	1.67	3.494	3.409	1.74	8.212	8.209	0.03
JL-1	Changchun, Jilin	17.789	4.916	4.856	0.87	4.413	4.377	0.58	7.952	7.803	1.34
JL-2	Changchun, Jilin	11.299	3.759	3.721	0.72	3.399	3.332	1.41	8.652	8.557	0.78
JL-3	Changchun, Jilin	12.483	2.698	2.707	0.24	4.365	4.402	0.60	6.685	6.774	0.94
JX-1	Bozhou, Jiangxi	11.812	3.689	3.639	0.96	3.098	3.045	1.22	8.741	8.703	0.31
JX-2	Bozhou, Jiangxi	15.536	3.925	3.991	1.18	3.403	3.439	0.74	8.839	9.046	1.64
JX-3	Bozhou, Jiangxi	16.379	1.913	1.881	1.19	4.347	4.296	0.83	6.926	6.737	1.96
JX-4	Bozhou, Jiangxi	18.735	3.838	3.803	0.65	4.412	4.384	0.45	5.935	5.878	0.68

t_{Rs} is the retention time of the reference and t_{Ri} is the retention time of other components.

4. Experimental Results

4.1. System Adaptability Test. 10 μL of mixed reference substance solution and 10 μL of test substance solution under item "2.2" were accurately sucked and analyzed according to the chromatographic conditions under item "2.1." The chromatographic diagram is shown in Figure 2. Under the abovementioned chromatographic conditions, the theoretical plate number of the four components was more than 3000, the separation degree of the adjacent chromatographic peaks was more than 1.5, and the peak shape was good.

4.2. Investigation of Linear Relationship. The linear regression results of protocatechuic acid, catechin, quercetin, and luteolin are shown in Table 2, which shows that each component has a good linear relationship within the respective mass concentration range.

4.3. Precision Test. The RSD values of protocatechuic acid, catechin, quercetin and luteolin peak areas were 1.95%, 1.98%, 1.34% and 1.83% respectively, indicating that the instrument had good precision.

4.4. Repeatability Test. The average mass fractions of protocatechuic acid, catechin, quercetin, and luteolin were 3.704, 3.394, 16.473, and 8.848 g/mg, respectively. The RSD values were 1.97%, 1.99%, 1.76%, and 1.93%, respectively.

4.5. Stability Test. The RSD of mean mass fractions for protocatechuic acid, catechin, quercetin, and luteolin were 1.88%, 1.76%, 1.53%, and 1.81%, respectively, indicating that the solution was stable for 12 h.

4.6. Sample Addition Recovery Test. The recovery and RSD values of protocatechuic acid, catechin, quercetin, and luteolin were calculated. The results are shown in Table 3, which shows that the method has good accuracy.

4.7. Calculation of Relative Correction Factor f. The relative correction factors of quercetin to protocatechuic acid, catechin, and luteolin were calculated, and the average value was taken. The results showed that (Table 4), taking quercetin as a reference, the relative correction factors of protocatechuic acid, catechin, and luteolin were 1.1992, 0.8613, and 1.6069, respectively, with RSD less than 2.0%.

4.8. Investigation of Durability

4.8.1. Influence of Different Instruments on f. The relative correction factors of quercetin to protocatechuic acid, catechin, and luteolin were calculated, and the average value was taken. The results showed that (Table 5) the RSD values were all less than 2.0%. It shows that different HPLCs have no significant effect on the relative correction factor.

4.8.2. Influence of Different Chromatographic Columns on f. The relative correction factors of quercetin to protocatechuic acid, catechin, and luteolin were calculated, respectively, and the average value was taken. The results showed that (Table 6), RSD values were all less than 2.0%. It shows that different chromatographic columns have no significant effect on the relative correction factor.

4.8.3. Influence of Different Volume Flowrates on f. The relative correction factors of quercetin to protocatechuic acid, catechin, and luteolin were calculated, and the average value was taken. The results showed that (Table 7) the RSD values were all less than 2.0%. It shows that different volume flow rates have no significant effect on the relative correction factor.

4.8.4. Influence of Different Column Temperatures on f. The relative correction factors of quercetin to protocatechuic acid, catechin, and luteolin were calculated, and the average value was taken. The results showed that (Table 8) the RSD values were all less than 2.0%. It shows that different column temperatures have no significant effect on the relative correction factor.

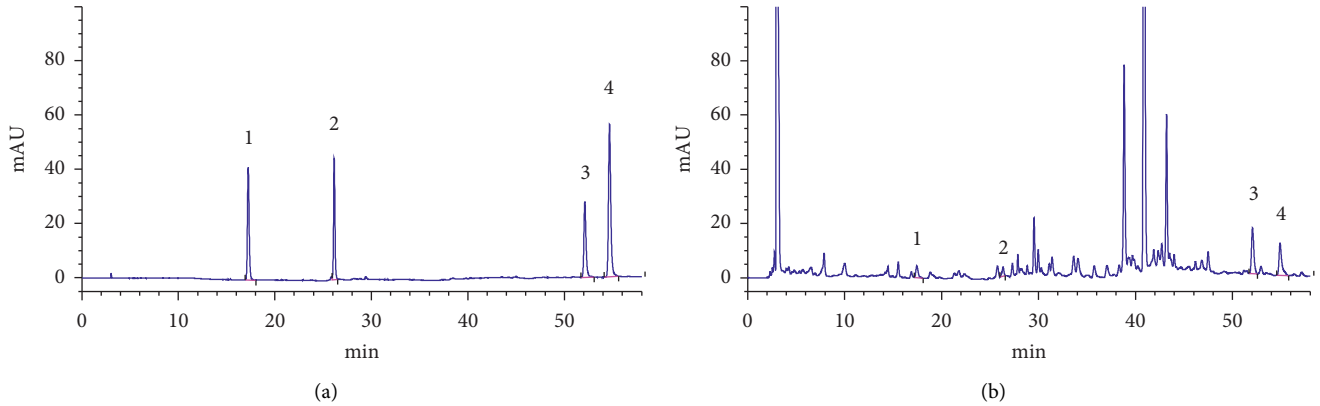


Figure 2: 1-protocatechuic acid, 2-catechin, 3-quercetin, and 4-luteolin. (a) Chromatogram of mixed control substance. (b) Chromatogram of *Plantago depressa* sample for test.

Table 2: Linear relationship of components.

Component	Regression equation	Linearity range/(μg/mL)	r
Protocatechuic acid	$Y = 19.36X + 30.992$	0.740 ~ 37.000	0.9996
Catechin	$Y = 11.695X + 55.552$	0.678 ~ 33.900	0.9998
Quercetin	$Y = 17.105X - 5.704$	3.292 ~ 164.600	0.9999
Luteolin	$Y = 27.728X - 27.397$	1.768 ~ 88.400	0.9995

Table 3: Recovery results of sample addition for each component ($n = 6$).

Component	Original/μg	Addition/μg	Measured/μg	Recovery rate/%	Average recovery rate/%	RSD/%
Protocatechuic acid	9.173	10.04	19.10	98.85	100.38	1.84
	9.554	10.04	19.53	99.31		
	9.713	10.04	19.73	99.80		
	9.228	10.04	19.67	104.00		
	9.376	10.04	19.43	100.10		
	9.253	10.04	19.32	100.23		
Catechin	8.843	9.10	17.80	98.42	97.38	1.74
	8.446	9.10	17.12	95.26		
	8.375	9.10	17.19	96.81		
	8.610	9.10	17.36	96.20		
	8.603	9.10	17.71	100.03		
	8.226	9.10	17.10	97.54		
Quercetin	41.705	41.20	82.02	97.86	99.14	1.69
	40.359	41.20	82.07	101.25		
	42.005	41.20	82.08	97.27		
	41.917	41.20	82.97	99.65		
	42.532	41.20	82.90	97.98		
	40.277	41.20	81.82	100.82		
Luteolin	24.998	25.10	49.22	96.51	97.66	1.47
	25.253	25.10	49.40	96.22		
	25.071	25.10	49.73	98.25		
	24.774	25.10	49.92	100.16		
	25.163	25.10	49.53	97.10		
	25.275	25.10	49.81	97.73		

4.9. Locations of Chromatographic Peaks of Components to be Detected. The relative retention values of quercetin to protocatechuic acid, catechin, and luteolin were calculated, and the average values were taken. The results showed that (Table 9) the RSD values were all less than 2.0%. The results show that the relative retention values of each component to be detected have good reproducibility in different high-performance liquid chromatography systems and chromatographic columns.

Table 4: Relative correction factors.

Dilution ratio	Relative correction factor		
	Protocatechuic acid	Catechin	Luteolin
1	1.204	0.8655	1.6158
2	1.1789	0.8342	1.5792
4	1.2062	0.8719	1.6203
5	1.2115	0.8803	1.6308
10	1.1923	0.8554	1.5831
20	1.2022	0.8602	1.6119
Mean	1.1992	0.8613	1.6069
RSD/%	0.98	1.84	1.30

Table 5: Influence of different instruments on relative correction factors.

Chromatographic instruments	Relative correction factor		
	Protocatechuic acid	Catechin	Luteolin
Agilent 1260	1.2041	0.8655	1.6158
Shimadzu LC-2030	1.1988	0.8521	1.5722
Mean	1.2097	0.8677	1.6089
RSD/%	1.19	1.65	1.45

Table 6: Effects of the different chromatographic columns on the relative correction factor.

Chromatographic columns	Relative correction factor		
	Protocatechuic acid	Catechin	Luteolin
Agilent ZORBAX SB-C_{18}	1.2035	0.8673	1.6139
Phenomenex C18	1.2041	0.8655	1.6158
Agilent TC-C_{18}	1.1962	0.8542	1.5902
Mean	1.2097	0.8677	1.6089
RSD/%	1.19	1.65	1.45

Table 7: Influence of different flow rates on relative correction factors.

Flow rates (mL/min)	Relative correction factor		
	Protocatechuic acid	Catechin	Luteolin
0.8	1.2105	0.8723	1.6337
0.9	1.1908	0.8604	1.5986
1.0	1.2041	0.8655	1.6158
1.1	1.2286	0.8902	1.6339
1.2	1.1874	0.8544	1.5771
Mean	1.2078	0.8720	1.6161
RSD/%	1.59	1.83	1.09

4.10. Comparison between the QAMS Method and the External Standard (ESM) Method. 10 batches of *Plantago depressa* with the proper amount from different producing areas were taken, with 3 copies for each sample. They were weighed precisely, and the solution for testing products was prepared according to item "2.2.2." The determination was carried out according to this method, and the chromatographic peak area of the components to be tested was recorded.

Table 8: Influence of different column temperatures on relative correction factors.

Column temperatures (°C)	Relative correction factor		
	Protocatechuic acid	Catechin	Luteolin
20	1.1965	0.8603	1.5971
25	1.2022	0.8647	1.6025
30	1.2241	0.8755	1.6258
35	1.1963	0.8599	1.5998
40	1.2138	0.8671	1.6123
Mean	1.2075	0.8667	1.6094
RSD/%	1.21	0.92	0.89

10 μL of each sample solution was precisely sucked and injected into high-performance liquid chromatography for determination. The content of quercetin, protocatechuic acid, catechin, and luteolin in the samples was calculated by the QAMS and ESM methods. The results are shown in Table 1. The results showed that the RSD values of the two methods were all within 2.0%, indicating that there was no significant difference in the contents of each batch of samples measured by the two methods, and the QAMS method could be applied to the determination of each component in *Plantago depressa*.

5. Discussion

In this study, the content of quercetin in *Plantago depressa* was determined by HPLC with an external standard method, and the relative correction factors of protocatechuic acid, catechin, and luteolin were calculated. Then, the content of protocatechuic acid, catechin, and luteolin was calculated with the obtained relative correction factors, thus realizing the quantitative analysis of multicomponents by single marker (QAMS). The content of three components in *Plantago depressa* was determined by an external standard method, and the difference between them and those calculated by the relative correction factor was compared. The results showed that there was no significant difference between the content values calculated by the relative correction factor and those determined by the external standard method. The method can improve the quality control of *Plantago depressa* without an increase of cost and can be used for quantitative analysis and quality evaluation of *Plantago depressa*.

5.1. Selection of Detection Wavelength. Xiao Jin et al. [26] used the HPLC method to simultaneously detect the content of 10 flavonoids in 16 *Acanthopanax* Miq. plants, of which quercetin content was detected at 283 nm. Li Jun et al. [27] used 260 nm and 360 nm dual wavelength switching methods to determine protocatechuic acid, quercetin, and luteolin in *Aspidistra lurida*. Que Zuliang et al. [28] chose a 225 nm detection wavelength when identifying catechin in the HPLC fingerprint of *Angelica sinensis*. Referring to the abovementioned literature, the absorption peaks at 225 nm, 260 nm, 283 nm, and 360 nm were simultaneously collected by using 190 nm ~ 400 nm full wavelength scanning and a

Table 9: Influence of different chromatographic columns and instruments on relative retention time.

Chromatographic instruments	Chromatographic columns	Relative retention time		
		Protocatechuic acid	Catechin	Luteolin
Agilent 1260	Agilent ZORBAX SB-C_{18}	0.3308	0.5017	1.049
	Phenomenex C18	0.3342	0.5128	1.0609
	Agilent TC-C_{18}	0.3285	0.4996	1.0428
Shimadzu LC-2030	Agilent ZORBAX SB-C_{18}	0.3317	0.5027	1.0634
	Phenomenex C18	0.3342	0.5101	1.0572
	Agilent TC-C_{18}	0.3215	0.4988	1.0373
Mean		0.3302	0.5043	1.0518
RSD/%		1.44	1.15	0.99

DAD detector. The maximum absorption wavelength of protocatechuic acid, catechin, quercetin, and luteolin was determined to be 225 nm.

5.2. Selection of Internal Standard. Quercetin is one of the active components of *Plantago depressa*, which has anti-inflammatory, antibacterial, antipathogenic microorganisms, antiviral, and other pharmacological effects. Due to its economic price, the simple preparation method and comprehensive consideration of the peak time and content of each component to be tested in quercetin, quercetin is finally determined as the internal standard.

5.3. Selection of Chromatographic Conditions. In this study, the effects of methanol-water, methanol-0.1% phosphoric acid solution, acetonitrile-water, and acetonitrile-0.1% phosphoric acid solution on the separation of four components in *Plantago depressa* were investigated. Through experiments, it is found that if the composition of the mobile phase is methanol-water and acetonitrile-water, the fluctuation of the baseline increases, so this option is excluded. When water is replaced with a 0.1% phosphoric acid solution, this situation can be effectively improved, and the baseline is relatively stable. However, the mobile phase system of methanol-0.1% phosphoric acid solution has a better peak shape than that of the acetonitrile-0.1% phosphoric acid solution system, so the mobile phase system is determined to be methanol-0.1% phosphoric acid solution. In this study, four different column temperatures such as 25°C, 30°C, 35°C, and 40°C and four different injection amounts such as 5 μL, 10 μL, 15 μL, and 20 μL were also investigated. Based on the combination of peak time, separation effect of chromatographic peaks to be detected, and economic factors, the chromatographic column temperature of 30°C, and injection amount of 10 μL were finally determined.

6. Conclusion

In this study, the content of quercetin in *Plantago depressa* was determined by HPLC with an external standard method, and the relative correction factors of protocatechuic acid, catechin, and luteolin were calculated. Then, the content of protocatechuic acid, catechin, and luteolin was calculated with the obtained relative correction factors, thus realizing the quantitative analysis of multicomponents by single marker (QAMS). The content of three components in *Plantago depressa* was determined by an external standard method, and the difference between them and those calculated by the relative correction factor was compared. The results showed that there was no significant difference between the content values calculated by the relative correction factor and those determined by the external standard method. The method can improve the quality control of *Plantago depressa* without an increase of cost and can be used for quantitative analysis and quality evaluation of *Plantago depressa*.

Data Availability

The main tables and figure data used to support the findings of this study are included within the article.

Ethical Approval

Ethical Approval is not applicable for this article.

Consent

There are no human subjects in this article and informed consent is not applicable.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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